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14. ABSTRACT

The purpose of this study is to determine the stability, elution profiles and pharmacodynamic properties, in bone cement, of available antibiotic and antifungal medications in an effort to establish a reference of local antibiotic delivery options for treating osteomyelitis and contaminated open fractures with emphasis on viable antibiotic combinations in bone cement for the treatment of multiple drug resistant organisms. The project has successfully developed analytical chemistry methods for description of *in vitro* antibiotic elution profiles that will be useful when analyzing elution profiles of bone cement containing multiple antibiotics. Early results have successfully identified previously unknown elution potential in bone cement for antibiotics of interest in treating contaminated war wounds and antibiotic resistant infections. In addition, early results contest the historical belief that liquid antibiotics are not compatible with bone cement for purposes of local antibiotic treatment. Analytic chemistry evaluation is currently being completed on the last remaining antibiotic bead sets for individual antibiotics/antifungals and bacterial susceptibility for viable beads is set to begin.

15. SUBJECT TERMS
antibiotic beads, contaminated wounds, multidrug resistant bacteria

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INTRODUCTION.

Local antibiotic delivery via bone cement is an important adjunct in the treatment of osteomyelitis and severe open fractures. The body of literature addressing antibiotic impregnated bone cement largely deals with options for pathogens typically encountered in endoprosthetic infections. In today's era of emerging multiple drug resistant bacteria and atypical infections encountered in severe open fractures and contaminated war wounds, there is a need for further guidance on available antibiotic bone cement options for the orthopaedic trauma surgeon. The purpose of this study is to determine the stability, elution profiles and pharmacodynamic properties in bone cement of available antibiotic and antifungal medications in an effort to establish a reference of local antibiotic delivery options for treating osteomyelitis and contaminated open fractures. Emphasis will be directed towards establishing viable antibiotic combinations in bone cement for the treatment of multiple drug resistant organisms encountered in severe open fractures and contaminated war wounds.

BODY.

Work on this project was delayed due to the pending transfer of the award from the TRUE Research Foundation to The Geneva Foundation. After this initial delay, the project began in May 2007 with the methods carried out via subcontract with the San Diego State University (SDSU) Department of Chemistry. Subcontract work by SDSU required hire of several laboratory assistants, which was done with success by SDSU in the spring of 2007. However, due to circumstances at SDSU the initial trained laboratory assistants were unable to continue as employees of SDSU and subsequently left the project before antibiotic analysis began. SDSU was able to hire and retrain appropriate laboratory assistants and by October 2007 analysis of antibiotic beads was underway. Subsequently, a no cost extension was requested for this project which was granted. Despite initial delay, substantial progress has been made on this project. Antibiotics with previously unknown elution potential in bone cement have been identified that may be of use in treating open war wounds and multiple drug resistant bacteria. Furthermore, the previously held belief that liquid antibiotics can not be incorporated into bone cement and retain elution potential has been dispelled.

OBJECTIVE 1: To develop a comprehensive reference of antibiotic and antifungal medications for inclusion in bone cement for the purposes of local delivery to tissues (Months 1-12):

Work is nearing completion evaluating elution properties of individual antibiotic and antifungal medications in bone cement for purposes of local delivery to tissues. Emphasis of this project is on antibiotics for local treatment of multiple drug resistant organisms commonly seen in contaminated war wounds and severe open fractures. We sought to establish antibiotic bone cement via clinically relevant means in that most antibiotic beads are prepared via the acquisition of pharmaceutical stock and prepared by the surgeon at time of surgery. Therefore, the forms and quantities of antibiotics used in bead preparation were from pharmaceutical preparations found in hospital pharmacies and also potentially found in forward deployed military treatment facilities.

Each testing sample of antibiotic was thoroughly mixed with Palacos polymethylmethacrylate cement powder prior to addition of monomer liquid. The prepared bone cement was extruded into a standard antibiotic bead mold in an effort to control for surface area size of all beads. No vacuum or centrifuging techniques were employed to form the cement in an effort to reproduce the clinical environment these beads would likely be prepared in. The weight and volume of each bead set was recorded before elution analysis. Each bead sample was submerged in a measurable amount of sterile phosphate buffered saline (PBS) solution (pH 7.4) and maintained at 37 deg C.

Analysis and quantification of the medications was to be performed using the highly sensitive and selective method of liquid-chromatography-tandem mass spectrometry (LC-MS). This method was chosen because it typically provides unambiguous quantitative results over a wide concentration range with excellent sensitivity, typically between 1 and 25 ng/mL for antibiotic-like compounds. In addition, it typically can be used to identify sample breakdown products that may form. It was anticipated that the assays of each medication would require some method development to obtain reliable and quantifiable results. Each medication was expected to have unique chemical properties and thus these properties would dictate method specifics for individual antibiotics.

However, once analysis was begun using LC-MS it became obvious that many of the antibiotics of interest were ionic in nature with the PBS salts interfering with analysis of the antibiotic compounds. Significant issues with antibiotic degradation occurred when running samples on the LC-MS. Methods were revised numerous times and insight gained that allowed generalized procedures for testing samples:

1. Antibiotics of lower molecular weight that are not highly soluble in water may be separated from interfering salt by HPLC procedures. If they are below 1500 molecular weight, they can be run by the best techniques we have- LC-MSMS methods. These antibiotics will have the highest likelihood for successful analysis.
2. Antibiotics of low molecular weight that are highly water soluble need to be separated by prior extraction techniques before analysis. Normal reverse phase solid phase extraction techniques do not work for these compounds.
3. Antibiotics that are above a molecular weight of 1500 have to be free of salts to obtain high quality analysis. If the antibiotics are ionic compounds that are made highly soluble by the addition of sulfuric or phosphoric acid, they need to be cleaned up by ion exchange chromatography.
4. Antibiotics with a molecular weight above 2000 will be difficult to analyze. Analysis is possible to as high as 4000 molecular weight but at reduced sensitivity.

Due to the issues encountered when evaluating antibiotics via LC-MS a large portion of antibiotics are being analyzed using UV Spectroscopy to allow for successful examination of elution profiles. The beads were handled with latex gloves and sterilized under UV lamp for about 5 minutes. Previously autoclaved 250 mL Erlenmeyer flasks were weighed and then the beads were transferred to each one and reweighed. To each flask 150 mL sterile PBS solution was added and then caps were sealed. The flasks were placed on mechanical shaker at 37°C with 100 rpm Starting from day 0, 1

mL aliquot was withdrawn from each flask at every time point and the uv-vis spectrum was taken between 400 – 200 nm. Appropriate dilutions were made whenever required.

At this time elution analysis has been completed on a total of 16 different antibiotic bead sets. (Table 1) Both liquid and solid forms of antibiotics yielded meaningful data. Oral suspensions proved to be too challenging to isolate and recognize in the phosphate buffered elution solution due to ingredients of the suspension complicating detection of the medication of interest. This leaves a total of 21 outstanding bead sets of individual antibiotics/antifungals that are currently being analyzed to complete Objective 1. (Table 2)

With the current available data several reportable findings have been made. Antibiotic elution potential in bone cement for antibiotics of interest in treating Acenitobacter *sp* bacteria have been identified in this study. Specifically, Linezolid and Doxycycline have displayed reliable elution from bone cement beyond 21 days. These findings have not been reported to date. In addition, antibiotics in liquid form were used in this study to prepare antibiotic beads and elution profiles for beads prepared with liquid Sulfamethoxazole:Trimethoprim and liquid Clindamycin displayed reliable elution properties beyond 21 days. This finding dispels a previous “standard” for antibiotic bead preparation in that only solid form antibiotics are capable of forming antibiotic beads with elution potential.

Bacterial susceptibility for prepared bead sets is gearing up to begin with the recent acquisition of a dedicated incubator for this portion of the study. It will be important to evaluate potential effectiveness of all bead sets regardless of analytical chemistry elution data. Appropriate bacteria *sp* have been purchased and are awaiting delivery upon initiation of incubator function.

With completion of Objective 1 this study will be ready to move into Phase 2 in an effort to achieve Objective 2 which is to establish viable antibiotic combination options for local delivery to tissues via bone cement when treating multiple drug resistant organisms. With completion of Objective 1 no new analytical chemistry methods for elution analysis are anticipated to be needed in that all antibiotics of interest will have established standards previously identified and analyzed during work on Objective one. We anticipate this fact to decrease the expected time to complete this study thus allowing the project to finish as planned at the end of year two.

KEY RESEARCH ACCOMPLISHMENTS

- Development of analytical chemistry methods for description of *in vitro* antibiotic elution profiles that will be useful when analyzing elution profiles of bone cement containing multiple antibiotics.
- Identified previously unknown elution potential in bone cement for antibiotics of interest, linezolid and doxycycline, in treating contaminated war wounds and antibiotic resistant infections with special interest in treating Acenitobacter *sp*.
- Identified previously unknown elution potential for liquid antibiotics mixed in bone cement as proven by Bactrim and Clindamycin bead sets prepared with liquid antibiotics. This finding dispels a currently held belief that only solid form antibiotics are capable of providing elution of antibiotic in antibiotic beads.

REPORTABLE OUTCOMES.

No manuscripts, presentations, patents, or licenses have resulted from this project to date. There will be two abstracts submitted to the 2008 Society of Military Orthopaedic Surgeons Annual Meeting from this project:

1 IDENTIFICATION OF UNRECOGNIZED ANTIBIOTIC BONE CEMENT OPTIONS FOR TREATING ACINETOBACTER sp
2 EXAMINING A TENET OF ANTIBIOTIC BONE CEMENT PREPARATION: LIQUID ANTIBIOTICS CAN WORK IN LOCAL DELIVERY VIA BONE CEMENT.

To date no outcomes have been reported in any fashion for this project. In addition, no further funding has been requested for projects related to this project. The Investigators have nothing to disclose at this time concerning reportable outcomes.

CONCLUSION.

The purpose of this study is to determine the stability, elution profiles and pharmacodynamic properties in bone cement of available antibiotic and antifungal medications in an effort to establish a reference of local antibiotic delivery options for treating osteomyelitis and contaminated open fractures. Substantial progress has been made on this project despite delay in beginning data collection. Antibiotics with previously unknown elution potential in bone cement have been identified that may be of use in treating open war wounds and multiple drug resistant bacteria. Furthermore, the previously held belief that liquid antibiotics can not be incorporated into bone cement and retain elution potential has been dispelled. This project has developed analytical chemistry methods for description of in vitro antibiotic elution profiles that will allow successful completion of the first year of work and will be useful when analyzing elution profiles of bone cement containing multiple antibiotics.

SUPPORTING DATA

TABLE 1: COMPLETED ANTIOBITIC BEAD SET ANALYSIS

Cement Bead #	Antibiotic	Concentration	Antibiotic Form	Antibiotic Detected	Elutes > 21 days
1	Imipenem:Cilastin	500:500mg	Solid	Yes	Yes
2	Levofloxacin	500mg	Solid	Yes	No
3	Levofloxacin	500mg/20mL	Liquid	Yes	No
4	Sulfamethoxazole:Trimethoprim	400:80mg	Liquid	Yes	Yes
5	Doxycycline	100mg	Solid	Yes	Yes
6	Ciprofloxacin	400mg/40mL	Liquid	Yes	No
7	Ciprofloxacin	400mg	Solid	Yes	Yes
8	Colistin	150mg	Solid	No	No
9	Clindamycin	900mg/6mL	Liquid	Yes	Yes
10	Daptomycin	500mg	Solid	No	No
11	Linezolid	3 grams	Solid	Yes	Yes
12	Ceftriaxone	2g	Solid	Yes	No
13	Oxacillin	2g	Solid	Yes	Yes
14	Amphotericin B	50mg	Solid	No	No
15	Meropenem	1000mg	Solid	Yes	No
16	Gatifloxacin	400mg	Liquid	Yes	No

TABLE 2: BEAD SETS CURRENTLY BEING ANALYZED

ANTIBIOTIC	ANTIBIOTIC FORM
Rifampin	Solid
Aztreonam	Solid
Ampicillin/Sulbactam (Unasyn)	Solid
Azithromycin	Solid
Caspofungin Acetate	Solid
Ceftazidine	Solid
Tobramycin Sulfate	Solid
Tobramycin Sulfate	Liquid
Amikacin Sulfate	Liquid
Cefepime	Solid
Dalfopristin	Solid
Metronidazole	Solid
Minocycline	Solid
Ticarcillin	Solid
Tygacil	Solid
Moxifloxacin	Solid
Amikacin	Liquid
Zosyn	Solid
Sulfadiazine	Solid
Lincomycin	Liquid
Sulfamethoxazole/Trimethoprim	Solid